REMARKS

The examiner's agreement that the claims now meet the requirements of 35 USC 112 first paragraph is appreciated. This leaves only the issue of whether the claims meet the non-obviousness requirement of 35 USC 103.

There are apparently two arguments being made: one based on Saiko in view of Davis; the other based on a combination of Walles, Yorke, Trubinkova and Davis.

So far as the first of these is concerned, the applicant has previously pointed out the differences in ovulation between various animals and that such differences affect the usefulness of data from one animal species in predicting activity in another. The examiner responds by noting that the specification indicates that some animal models may be of use and that the specification contains only prophetic material.

In response to these comments, the applicant points out the following and supplies some relevant data.

Animals fall into two main groups for consideration of ovulation: reflex and non-reflex (spontaneous) ovulators. Mammalian females can be broadly categorized as either spontaneous or reflex (induced) ovulators. Spontaneous ovulatory cycles (ie., estrous or menstrual) occur independent of coitus; this system is controlled internally by interrelated oscillations in balance of steroid, hypothalamic and pituitary hormones. Reflex ovulators are induced to ovulate as a direct neural response to mating. Reflex ovulators includes rabbits, ferrets, mink, voles, camels, domestic cats, the short-tailed tree shrew, and 13-lined ground squirrel. Spontaneous ovulators include primates (including humans) and dogs. For more information see:

Neuroendocrinology at

http://www.uwyo.edu/wjm/Repro/neuro.htm

and

The Physiology of Canine Reproduction at

http://74.125.45.132/search?q=cache:obVnfCBXPNkJ:www.seefido.com/html/the_physiology_of_canine_repro.htm+dog+%22spontaneous+ovulation%22&hl=en&ct=clnk&cd=1&gl=us

These differences in mechanism result in different hormonal effects on ovulation and in particular different causes for release of gonadotrophin releasing hormone. Thus rabbit data are not appropriate for predicting effects in humans. The primary reference relied upon (Saiko) relates to studies in rabbits which are reflex ovulators. Humans are spontaneous ovulators. The examiner comments that only prophetic examples have been provided. The following data, obtained from experiments with dogs, which like humans are spontaneous ovulators, is submitted in support of the applicant's contention that the invention as claimed is not obvious.

Animal Data

Thirty-nine female beagle dogs, aged 7 to 9 months, were administered galanthamine hydrobromide once daily by capsule, in doses of 0, 2, 5 and 10 mg/kg. The doses were chosen, based on a pilot study, to produce marked cholinergic symptoms (10 mg/kg), to be essentially asymptomatic (2 mg/kg), or to be intermediate (5 mg/kg). Good Laboratory Practices were followed. (Chau RY, Nagae Y, Yau ET, Galanthamine Hydrobromide 26/52-week oral toxicity study in dogs, Research Department, Pharmaceuticals Division, Ciba-Geigy Corporation, Summit, New Jersey, March 29, 1994).

Side effects occurred. At all dose levels, cholinergic side effects consisting of fasciculations, hyperactivity, lacrimation, salivation, tremors, fecal changes including diarrhea and soft or mucoid feces occurred post-dosing. At $\geq$ 5 mg/kg, urinary incontinence and bloody feces occurred. At 10 mg/kg, ataxia, hypoactivity, excessive panting, nasal discharge, labored breathing, rigidity, loss of bowel control and emesis were seen.

Four animals at each dose level were sacrificed at 26 weeks. The estrus cycle was not monitored during life, and no information on the stage of the cycle was recorded at autopsy. Elevated uterine weights suggested that all of the control animals were in a stimulated phase of the cycle, and no apparent relationship existed with galantamine treatment. At 52 weeks, four additional animals from each dose level were sacrificed and their organs examined pathologically. The stage of the estrus cycle was noted. Animals in metestrus, the stage of the cycle during following estrus, during which corpora lutea form in ovaries which have undergone follicular development and ovulation, showed highly significant enhancement of ovarian weight by galantamine treatment (r=.848, p=.0039).

Inspection of Figures 1 and 2, ovary weights, and ovary weights as a percentage of brain weights, respectively, suggests ovarian stimulation even at the lowest, the 2 mg/kg dose.

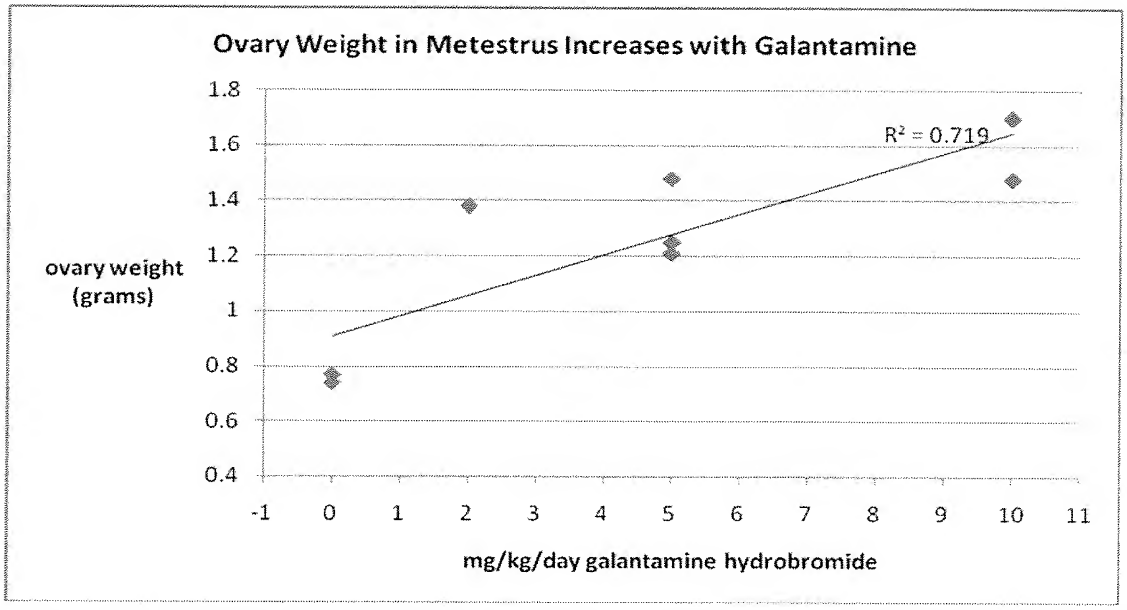

Figure 1. Ovarian weight in metestrus dogs treated with galantamine. Galantamine treatment for one year increased ovarian weights in dogs treated with galantamine. r=.848, p=.0039

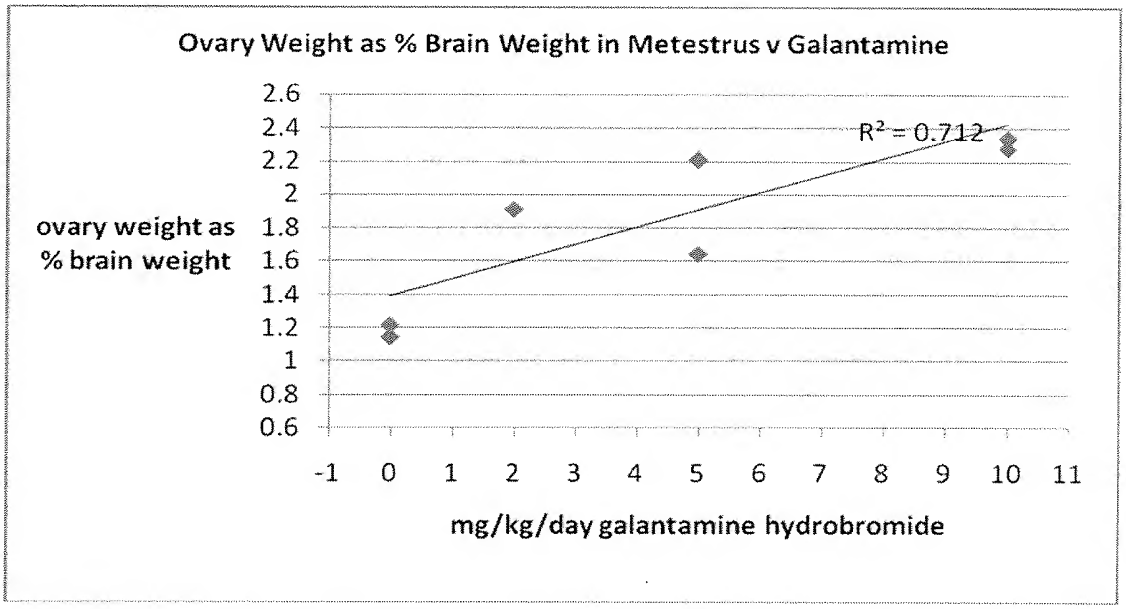

Figure 2. Ovarian weight in metestrus dogs treated with galantamine, expressed as percent brain weight. Galantamine treatment for one year in increased ovarian weights. r=.844, p=.0042

No significant effect of galantamine on ovarian weight was seen in animals who were sacrificed during the quiescent anestrus period, as shown in Figures 3 and 4.

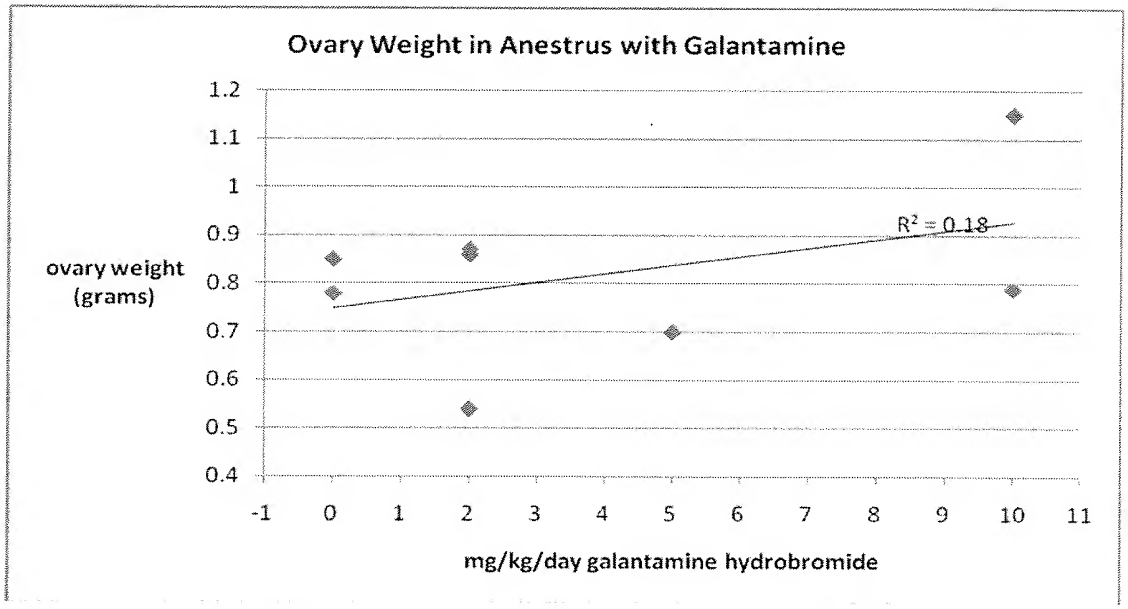

Figure 3. Ovarian weight in anestrus dogs treated with galantamine. No significant effect was seen after one year of treatment. r=.424, p=.2554

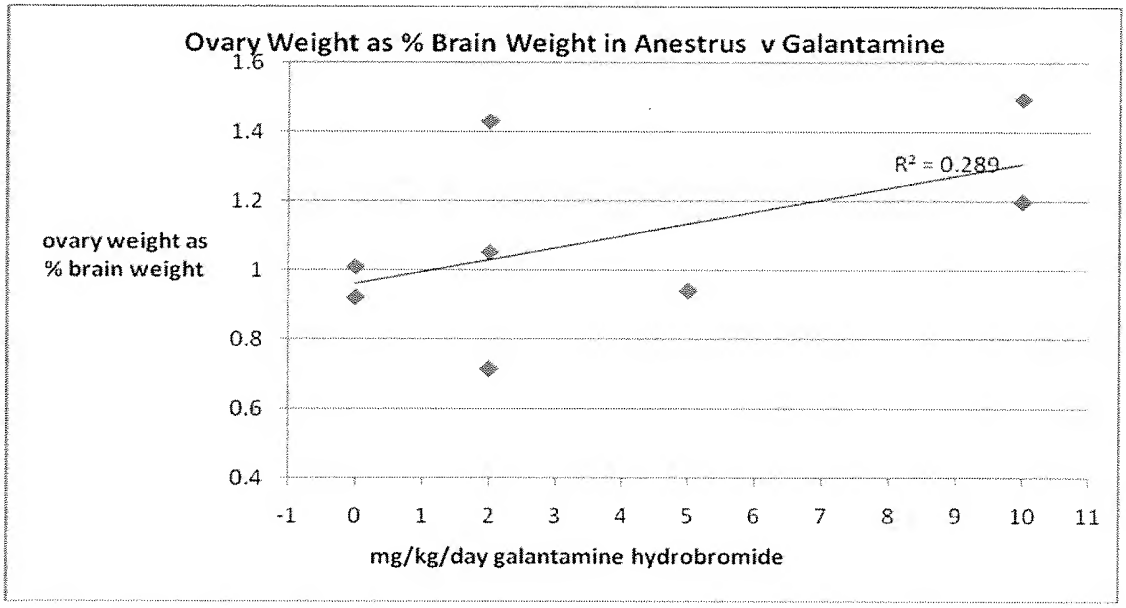

Figure 4. Ovarian weight as a percent of brain weight in dogs treated with galantamine. One year of treatment had no significant effect during anestrus. r=.538, p=.1351

Pathological examination of the 52 week animals confirmed ovarian stimulation by galantamine treatment. One of the four control animals, a dog in anestrus, had a unilateral ovarian luteal cyst.

None were seen in the 2 mg/kg group. A slight increase in the size or number of ovarian corpora lutea, and large ovaries, were observed in 1 of 4 females at 5 mg/kg, and in 2 of 4 females at 10 mg/kg, associated with pseudocyesis and endometrial hyperplasia. Statistically significant increases in uterine weights were apparent at dose levels ≥ 5 mg/kg. At 10 mg/kg, the uterus of one female was distended with fluid, and a tissue mass was noted in another.

Three control animals, and three females who had received 10 mg/kg for one year were sacrificed four weeks after the termination of one year's treatment. One of the high dose females had large ovaries with bilateral corpora lutea. The uterus contained a tissue mass and the diagnosis of moderate endometrial hyperplasia with pseudocyesis was made.

At the 52 week sacrifice, focal or multifocal degeneration of the tunica muscularis of the urinary bladder was seen in 2 animals at the 10 mg dose. This was not present in animals sacrificed after the four week recovery period and was not in the muscularis of other tissues.

It is clear from these data that galantamine treatment can stimulate ovarian growth and follicle development, to a greater extent at high doses than would be desirable in fertility enhancement.

Some comparison of the dosing to the proposed dosing for humans is possible. The 2 mg and 5 mg doses yielded peak plasma levels of 260±21 and 459±214 ng/ml of galantamine hydrobromide in female dogs. (Robertson P, Klerer CP, Galanthamine HBr: Analysis of plasma samples from a toxicology 26/52-week oral toxicity study in dogs, Research Department, Pharmaceuticals Division, Ciba-Geigy Corporation, Summit, New Jersey, March 9, 1994.) There was no detectable drug by 6 hours. Data from the 10 mg/kg dose suggest that the half life of galantamine in the female dog is less than 2 hours. In contrast, in non-elderly humans, the peak plasma concentration after a 12 or 16 mg tablet is 97.4±31.4 and 137±36 ng/ml of galantamine base, respectively, which are equivalent to about 122 and 171 ng/ml of galantamine hydrobromide. These are lower than the levels achieved with 2 and 5 mg/kg in the dog. But the half-life in non-elderly humans is 7.3±1.7 to 7.9±0.8 hours. Thus, the exposure to a dose of galantamine is more prolonged in the human. Furthermore, the animals were dosed once a day, and humans are dosed twice, or can use an extended-release formulation, both of which maintain plasma levels throughout the waking day. Marked

reproductive system changes occurred with a short, daily exposure to galantamine in dogs. Larger areas under the curve can be achieved at clinically acceptable doses in humans. These data indicate that galantamine, when administered repeatedly on a daily basis, can, in fact, stimulate the mammalian reproductive axis. Application to humans, as well as to animals, can provide a safe and effective addition or alternative to current treatments. (Roussel JD, Beatty JF, Koonce K, Gonadotrophin releasing hormone therapy in functional infertility of dairy cattle. Theriogeneology, 30(6): 115 -9, 1988.)

It is therefore submitted that one skilled in the art would not have been led by the knowledge supplied by Davis that galanthamine and its derivatives have acetylcholinesterase inhibiting properties to have any reasonable expectation that such compounds would be of use in stimulation of ovulation in humans simply because Saiko indicates that "augmented cholinergic processes apparently stimulate release of the egg from the follicle" in female rabbits. Rabbits ovulate in response to the mechanical stimulus of coitus. Humans ovulate in response to changes in hormonal balances. It is simply not possible to make predictions from species of one type to the other. Furthermore, there has been an imprecision in terminology which has resulted in references to the extrusion of the egg from the follicle being cited against the use of galantamine to promote clomiphene-responsive ovulation.

In the Applicant's response to the Examiner's restriction action, the Applicant wrote:

> "Applicant identifies the disease as clomiphene-responsive ovulation stimulation in women and for improvement of oligospermia or aesthenospermia in men. Support for this is found throughout the specification, *inter alia*, on page 1, lines 6-7, page 3, lines 14-15; page 9, lines 16-19, and page 11, lines 14-23."

In his April 1, 2004 response, the Examiner wrote:

> "Examiner notes two disease states were selected for examination on the merits, only one invention was to be selected, thus, Examiner will examine the first selected invention: ovulation failure."

The specification does not propose the use of acetylcholinesterase inhibitors for any cause of ovulation failure. In particular, in the Summary of Invention, the phrase

"failure of ovulation" is preceded by "conditions that can benefit from stimulation of the hypothalamic-pituitary-gonadal axis."

The word "ovulation" is used in the field to refer to both the extrusion of the egg, and more generally to the development of the follicle until extrusion can occur. "Ovulation induction" or stimulation, in humans, however, refers to treatments which begin at the beginning of the menstrual cycle, or the end of the preceding cycle. A chapter entitled "Mechanism of Ovulation," describes the development of the follicle during the menstrual cycle. (Irianni F, Hodgen GD, Mechanism of Ovulation, Endo Metab Clin North America 212(1), March, 1992, pp 19-39)

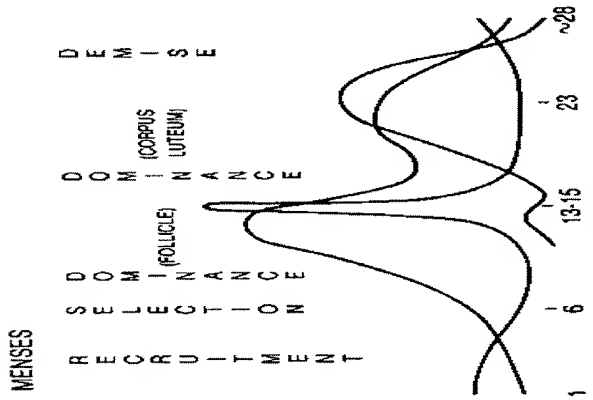

Figure 2. The terms used to describe the sequence of principal ovarian events during follicular maturation and CL function are temporally defined in the menstrual cycle. The curves depict idealized (stereotypical) patterns of E_2, pituitary gonadotropins, and P in peripheral circulation.

As shown in the chapter's Figure 2, and described in the chapter, the recruitment of follicles destined to develop to maturity begins with the onset of menses, and continues for 5-7 days. It is during this period that ovulation induction treatments designed to mimic hypothalamic and pituitary function are begun. According to Irianni and Hodgen,

> "Ovulation induction with clomiphene citrate or injectable gonadotropins is used commonly in the treatment of a wide variety of clinical disorders. It is well established that coordinating the onset and duration of these therapies with the status of the developing cohort of follicles is helpful in patient

management. Therapy is initiated during recruitment and is maintained for variable intervals.

"In contrast to clomiphene citrate, therapy with injectable gonadotropins is begun earlier in the follicular phase, typically on cycle day 2 or 3. [Clomiphene therapy begins day 2 to 5 of the menstrual cycle. (Blacker, p.64, previously submitted)] This, along with the increased concentrations of gonadotropins, results in ongoing development of multiple follicles. Initiating gonadotropin therapy later in the cycle decreases the number of quasisynchronous follicles available for recruitment. This is consistent with the declining number of follicles remaining in the cohort that have not begun the process of atresia. It should be noted however, that if gonadotropin levels are maintained sufficiently high other smaller follicles may be recruited as they enter the stage of gonadotropin sensitivity. These smaller follicles lag behind the follicles that were recruited earlier and are unlikely to produce fertilizable oocytes and thus provide no clinical benefit (Fig 7).

"The duration of gonadotropin administration in these patients is variable, and specific monitoring is required to direct therapy. The elevated gonadotropin concentrations tend to accelerate development, and follicular maturity becomes adequate to produce mature oocytes by approximately cycle day 12....

"Whether one should use the long [beginning day 20-24 of prior cycle, Fig. 8, p. 34]or short agonist/gonadotropin protocol [beginning day 2, Fig 8, p. 34] depends on many variables, including the stimulation regimen, monitoring system, patient population, clinician's expertise, and the protocol used in the egg/embryo laboratory. Data show that both can work either well or poorly. The long protocol is more versatile overall, and therefore is preferred by me." (Irianni and Hodgen, pp 34-35).

Blacker, provided earlier, administers clomiphene, which has a five day half-life, for five days "starting cycle day 2 to 5." (p 64) Human menopausal gonadotropin is "begun following spontaneous menses or induced withdrawal bleeding." (p 69) The initial "dose is administered daily for 3 days," and "increased every 2 to 3 days until a response is noted, " The accompanying Figure 2 from Irianni and Hodgen shows

treatment from days 3 to 11 of the menstrual cycle. When GnRH (also LRF) therapy is used, "most patients will ovulate after 10 to 20 days of pulsatile GnRH therapy." (p 75)

Thus, the stimulation of ovulation by medical means always begins with the promotion of maturation of the follicle by day 5 of the menstrual cycle, at least 9 days prior to the hoped-for ovulation, and the drugs or their effects continue for at least 10 days.

Turning now to the Examiner's second argument based on the combination of Walles, Yorke, Trubinkova and Davis, all of the references provided by the Examiner except for Davis refer to acute processes occurring at the time of extrusion of the egg. The Examiner's argument seems to be: Davis teaches certain compounds are acetylcholinesterase inhibitors; presence of acetylcholinesterase inhibitors is assumed to increase acetylcholine levels; acetylcholine causes contraction of human and cow follicles (Walles); contraction of follicles induces ovulation (Yorke and Trubinovka).

This argument rests on a number of assumptions and misapprehensions. First it ignores the fact that the claims are restricted to centrally acting cholinesterase inhibitors which would not be the compounds of choice for action local on the ovaries, which action is by definition peripheral in nature. Davis 6150354 teaches use of acetyl cholinesterase inhibitors for treatment of Alzheimer's disease. As noted at column 7 lines 50 -54, "To be effective [for treatment of Alzheimer's disease] , a compound must pass the blood brain barrier easily and distribute itself between the central and peripheral nervous systems in such a way that its effect is mainly central, and it must not have significant side effects" This is not a teaching that the compounds used would be compounds to choose for peripheral activity. The examiner's argument thus relies on the assumption that the centrally acting acetylcholinesterase inhibitors described by Davis will have a peripheral effect. There is no basis for this in the art cited. Walles studies involve direct application of acetylcholine to ovaries. One of the features of galanthamine and its analogs is that it partitions preferentially to the brain (claim 1 is limited to centrally acting cholinesterase inhibitors). There is therefore no reason to assume that such centrally acting compounds would result in a local increase in acetylcholine levels in the ovaries.

In any case, if even acetylcholine were a compound that facilitates egg extrusion, the notion that an egg extruder could solve ovulation problems which take at least ten days to address in any form of clinical intervention, is not plausible. It would make absolutely no sense to anyone in the field. Furthermore, if an egg at any stage were to be ejected from the follicle by cholinergic treatment, the protocols here, which begin when ova are immature, would destroy fertility.

Secondly, as the Examiner points out, "Walles et al. teaches that acetylcholine causes contraction in human and cow follicles," "Yorke et al. teaches that muscular contraction is [has been proposed as] associated with the expulsion of the egg from the follicle in [higher] vertebrates, and "Trubnikova et al teaches that contraction of follicular epithelium cells resulted in the retraction of the egg envelopes." Saiko cites changes in blood cholinesterase activities measured "1 hr after coitus." Thus Walles and Saiko associate acetylcholine with ovum extrusion within a period of an hour. If these statements have the significance attached to them by the Examiner, the problem of human female infertility could seemingly be solved by a single application of a medication at the time ovulation is desired. This is clearly not the case. Causing contractions of the egg follicles does not of itself result in human ovulation. However, even if it did there is no reason to expect that administration of a centrally acting cholinesterase inhibitor would cause such contractions.

Moreover, it is not clear that muscle stimulation alone could cause extrusion. Extrusion of a mature oocyte is thought to involve "proteolytic digestion of the follicular wall" by plasmin , other proteases, and collagenase. Prostaglandins, histamine, bradykinin participate in follicular rupture. Hyaluronic acid separates "the oocyte-cumulus complex from the granulosa membrane." And smooth muscle was postulated to "maintain a constant tension on the follicular wall, thereby assisting in ...the extrusion of the oocyte and follicular collapse." (Irianni and Hodgen) Thus, an effect of muscular tension, not even contraction, is merely postulated to be involved, and is, at most, a contributor to egg extrusion.

It is therefore submitted that the combination of Walles, Yorke, Trubinkova and Davis similarly does not provide any rational basis for administration of a centrally active cholinesterase inhibitor to treat failure of ovulation in humans.

If the references cited by the Examiner were sufficient to convince the field that cholinergicstimulation could expel the ovum, the continued application of cholinesterase inhibitors as proposed in the specification could be seen as interfering with fertility, expelling immature ova which cannot even be fertilized. The combination of art references cited by the examiner, if it were proper to read them together, would therefore point away from the present invention.

It is therefore submitted that the present claims meet the requirements of 35 USC 103 and should be allowed. An early action to this effect is respectfully solicited.

Respectfully submitted,

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1: Theriogenology. 1988 Dec;30(6):1115-9.

Gonadotropin releasing hormone therapy in functional infertility of dairy cattle.

Roussel JD, Beatty JF, Koonce K.

Department of Dairy Science Louisiana Agricultural Experiment Station Louisiana State University Agricultural Center Baton Rouge, LA 70803 USA.

Effects of gonadotropin-releasing hormone (GnRH) on conception rate was tested in 379 repeat-breeders in nine large dairy herds in Louisiana. Cattle with three or more services were treated intramuscularly with GnRH at the time of artificial insemination. The conception rate for the repeat-breeders treated with GnRH was significantly greater than for the controls (56 vs 40%). Furthermore, repeat-breeders that were treated with GnRH for two consecutive times at insemination resulted in a 53% increase in conception rate over the controls.

PMID: 17087900 [PubMed - in process]

Related Links

Conception rates in repeat-breeders and dairy cattle with unobserved estrus after
prostaglandin F(2) alpha and gonadotropin-releasing hormone. [Theriogenology. 1988] PMID:16726368

The reproductive performance of dairy cows with anovulatory anoestrus that were injected with either gonadotrophin-releasing hormone or oestradiol benzoate as part of a re-treatment process after insemination. [J S Afr Vet Assoc. 2007] PMID:17665758

Effect of gonadotrophin releasing hormone on conception rate in repeat-breeder dairy cows. [Theriogenology. 1986] PMID:16726226

Failure of gonadotropin-releasing hormone or human chorionic gonadotropin to enhance the fertility of repeat-breeder cows when administered at the time of insemination. [Theriogenology. 1990] PMID:16726895

Aquapuncture therapy of repeat breeding in dairy cattle. [Am J Chin Med. 2002] PMID:12230028

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MECHANISM OF OVULATION

Francisco Irianni, MD, and Gary D. Hodgen, PhD

The human ovarian follicle is a paradox of timeless and timely biological functions. The former because it can "sleep away" up to 5 decades before giving way to the latter, a fortnightly burst of growth and cell proliferation in preparation for ovulation.

However, neither of these fates is likely for any given ovarian follicle. Indeed, from a maximum of approximately 6 million ovarian follicles in the two gonads at the seventh month of intrauterine life, only about 2 million survive to reach neonatal life. By the time of menarche, this number has been depleted to only ~400,000 viable follicles. If a healthy woman who never becomes pregnant ovulates 13 times per year from the age of 15 to 50 years, less than 500 of the original 6 million ovarian follicles ever achieve ovulation. Thus, quantitatively and in terms of the probable fate of a given follicle, there is much to say about the process(es) of follicular atresia. Yet, the main focus of our remarks in this article are directed toward those few special follicles that somehow avoid the atretic mechanisms that constantly deplete the nonreplenishable store of ovarian follicles invested during fetal development.

There are at least two intervals when ovarian follicular growth is dependent on an appropriate supply of pituitary gonadotropins: first, during prenatal life when gonadal formation occurs; second, during adult ovarian/menstrual cycles when follicular maturation can attain ovulation. Certainly other growth-promoting hormones are necessary for follicle growth; however, these have been discussed in detail by

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other authors dealing with paracrine and, perhaps, autocrine cellular mechanisms.

When considering the temporal control of the ovarian cycle and in turn its regulation of the endometrium for the menstrual cycle, it is sometimes worthwhile to get a broader perspective. For example, the ovary has two functional roles that are distinct but intertwined. The first is hormonogenesis; the second is gametogenesis. Consider the reproductive strategy of our species. How can it be characterized? Typically, we bear our young in singleton pregnancies, and a woman's reproductive potential is spread over several decades, as opposed to many other mammalian species that may bear litters up to several times each year but over a shorter life span. Consider still further that only mammals have a luteal phase. Why so? Animals without a uterus do not need a corpus luteum (ie, birds and reptiles). The purposes of the ovarian follicle are to (1) preserve the resident oocyte; (2) mature the oocyte at the optimal time; (3) produce a hormonal milieu that will develop a lush proliferative endometrium; (4) yield well-timed release of the oocyte; (5) provide for high quality corpus luteum function, leading to implantation; and (6) preserve the hormonal conditions required for gestation until the fetoplacental metabolism is adequate—a formidable sequence designed by nature.

All of these points brings us to reconsider why the human ovarian/menstrual cycle is about 28 days, divided about equally into fortnightly follicular and luteal phases. But even this insight is fundamentally deficient, because it fails to explain how typically only one follicle achieves ovulation in each cycle. If both ovaries are perfused with the same blood supply—and therefore the same prevailing levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH)—why is it that typically only one ovary is gametogenically active, while the contralateral ovary is gametogenically quiescent? This happens because the total follicular pool in both ovaries acts in concert as a "unigonadal" feedback upon the hypothalamic-pituitary unit.

Another key question is when is the dominant follicle destined to be ovulated, that is, selected from among the growing cohort? Is it cued up in advance several months previously, or is it determined in the same ovarian/menstrual cycle in which it achieves ovulation? Whereas the answers are easy to provide now, it required several years of deliberate investigation to decipher the life cycle of the pelvic clock. We have employed laboratory primates for much of the invasive experimentation, because they too have a ~28 day ovarian/menstrual cycle. These studies have been addressed in various earlier reviews that favor either a more basic science or clinical orientation.[14, 29, 32]

Here, we hope to provide a fundamental basis for a better understanding of normal ovarian physiologic processes relevant to the pathophysiology of ovarian dysfunction, as well as clinical intervention to treat infertility or to achieve contraception. At the center of our attention will be the pelvic clock, which regulates by steroidal and nonsteroidal endocrine and paracrine messages both hypothalamic-pituitary and intraovarian functions essential to successful reproduction, and the steroidal milieu that generates the ~28 day human menstrual cycle.

FOLLICULAR FORMATION

On approximately day 24 of fetal life, germ cells arise in the yolk sac. They subsequently migrate to the gonadal ridge during the fifth week of development to form the indifferent gonad. These germ cells initially reside within nests without intervening stroma known as the cell syncytium. The migration of stroma between the oocytes marks the development of the primordial follicles.[33]

Nuclear maturation of the oocyte arrests in the diplotene stage of the first meiotic division. It will remain in this stage until ready to resume meiosis and potentially develop into a mature oocyte. The duration of this resting state, in which no further development occurs, may last for as little as a few days or as long as 50 years.

GONADOTROPIN-INDEPENDENT DEVELOPMENT

The earliest developmental changes that occur as the primordial follicles leave their resting state and resume development are independent of gonadotropin stimulation. Morphologically, the oocyte enlarges from approximately 15 μm to 80 to 100 μm, the zona pellucida is formed, and the surrounding granulosa cells proliferate to form two or more layers.[19] These follicles have no antrum and are considered to be primary follicles. This process occurs throughout the juvenile, prepubertal, and reproductive years through the climacteric and is unaffected by changes in circulating gonadotropin or sex steroid dynamics. This is demonstrated clinically in patients with olfactogenital dysplasia (Kallman's syndrome) who have a congenital deficiency of gonadotropin secretion and also in patients who are status posthypophysectomy.[26] In both circumstances, the normal process of primary follicular development appears unaltered.

Little is known about what initiates the resumption of folliculogenesis after many years in the resting state. It is most remarkable that one primordial follicle can resume development and proceed through ovulation, while another one, just microns away, may remain in the resting state for several decades more. It is clear, however, that this

process continues independent of the circulating gonadotropin and sex steroid milieu. Therefore, the departure of follicles from their resting state occurs throughout the prepubertal years, during pregnancy, and while on contraceptive steroids.[53] The lack of sensitivity of this process to known endocrine factors translates at the current time to an inability to clinically manipulate this portion of folliculogenesis.

TEMPORAL COORDINATION

The process of advanced folliculogenesis provides oocytes capable of fertilization while stimulating synchronous development of the remainder of the reproductive tract.[16] Precise coordination of these events is required if successful reproduction can occur. In humans, this means that typically only a single follicle containing a single oocyte usually reaches maturity and that the hormonal milieu created and controlled by this follicle induces precisely timed and specific changes within the cervix, fallopian tube, endometrium, and hypothalamic-pituitary axis.

The cyclic development of a single follicle with ovulation and corpus luteum function is much like a clock with predictable durations for each developmental stage. The follicles themselves coordinate the physiologic changes within the different parts of the reproductive system. Each has an intrinsically limited life span, with the decline of corpus luteum function eventually occurring in both conception and nonconception cycles.

GONADOTROPIN-DEPENDENT DEVELOPMENT

As the primary follicles mature, small loculi of fluid begin to form around the granulosa cells. As development progresses, these loculi coalesce and a fluid filled cavity or antrum is formed. Coincident with these changes is the acquisition of a theca layer, which is separated from the granulosa cells by the vascular lamina basalis. These morphologic changes hallmark the development of secondary follicles and the beginning of gonadotropin sensitivity.

The gonadotropin-sensitive interval of folliculogenesis has been studied more extensively and is better understood than the gonadotropin-insensitive portions. It is during this period that the classic morphologic and endocrine dynamics of folliculogenesis are well defined and that an understanding of these dynamics can lead to meaningful clinical intervention.

The fact that humans produce a single fertilizable oocyte appropri-

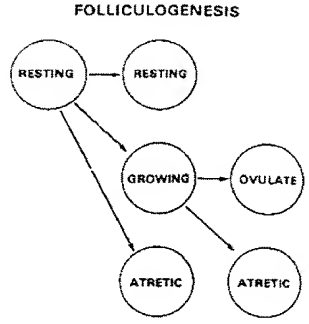

Figure 1. Ovarian follicles may be found in four basic conditions: at rest, growing atretic, or ready to ovulate.

ately every 4 weeks is well established. This is true despite the ability of follicles to leave the resting state and their potential to mature in a continuous process (Fig. 1). Therefore, there must be a means of stimulating adequate development of the follicle destined to ovulate while allowing all of the others to undergo atresia. The dynamics of this process have been characterized and divided into the intervals of recruitment, selection, dominance, and ovulation (Fig. 2).

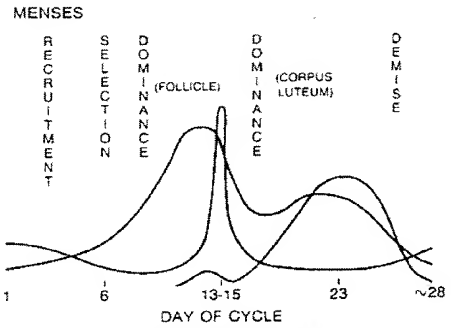

Figure 2. The terms used to describe the sequence of principal ovarian events during follicular maturation and CL function are temporally defined in the menstrual cycle. The curves depict idealized (stereotypical) patterns of E_2, pituitary gonadotropins, and P in peripheral circulation.

RECRUITMENT

As follicles enter the stage of gonadotropin sensitivity, they also become gonadotropin dependent. Inadequate gonadotropin stimulation during this critical period leads to atresia.[14] In normal menstrual cycles, circulating gonadotropins are too low throughout the majority of the cycle to stimulate progression of follicular development beyond this early stage (Fig. 3). However, as corpus luteal function fails at the end of a nonconception cycle, estradiol (E_2) and progesterone (P) levels decline and their suppression of the hypothalamic-pituitary axis decreases. This results in a narrow window during each monthly menstrual cycle in which the circulating milieu is favorable for rescue of these follicles so that further development might occur. This group of quasisynchronous follicles are referred to as a cohort and their early stimulation and development is termed recruitment (Fig. 4).[14]

During recruitment, multiple follicles are present that possess the ability to proceed to ovulation. These follicles are morphologically indistinguishable and ablation of any of them does not result in a delay in ovulation. As folliculogenesis progresses, they produce more E_2. The decline in FSH concentrations induced by the rising E_2 levels does not adversely affect the most mature follicles. In the process of developing, the follicles increase the number of FSH receptors and are capable of sustained growth even in the presence of lower FSH concentrations.[49] Other hormones probably involved in this process include gonadal peptides, such as inhibin, and possibly paracrine/autocrine hormones, such as a number of growth factors.

The process of recruitment begins at the end of the luteal phase of the prior cycle, from the onset of menses to approximately day 5 to 7 of the current cycle.[14] Eventually, only a single follicle will be able to utilize its hormonal milieu efficiently enough to sustain development until the interval of recruitment is completed.

SELECTION

Between day 5 and 7 of the normal 28 day cycle, a single follicle becomes destined to ovulate and form the corpus luteum. This is termed selection of the dominant follicle (Fig. 5). Selection is the culmination of the process of recruitment and marks the point in time at which the influence of a single follicle creates an environment in which only it can adequately mature and reach ovulation.

This has been demonstrated physiologically by experiments in which the largest follicle is ablated. If the ovaries are in the stage of recruitment, ablation of the most advanced follicle has little impact.

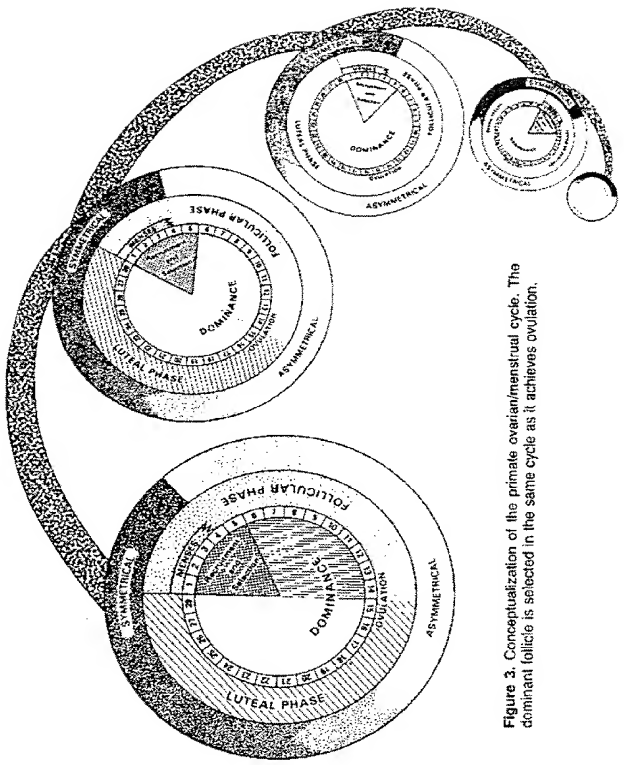

Figure 3. Conceptualization of the primate ovarian/menstrual cycle. The dominant follicle is selected in the same cycle as it achieves ovulation.

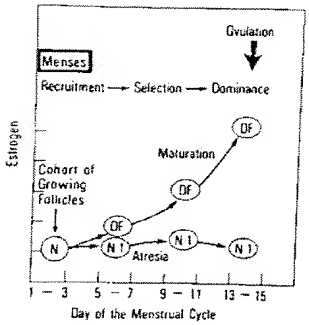

Figure 4. The course for recruitment, selection, and ovulation of the dominant ovarian follicle, with onset of atresia among other follicles of the cohort.

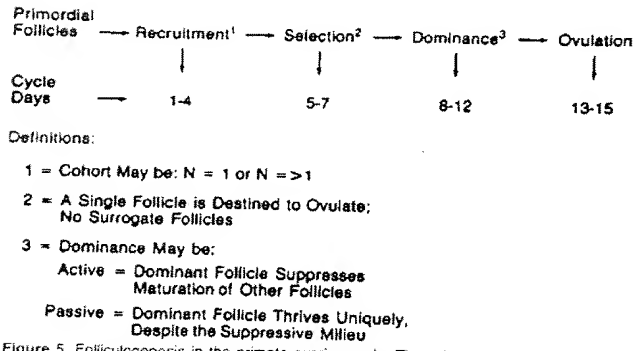

Figure 5. Folliculogenesis in the primate ovarian cycle. The cohort of follicles from which the ovulatory follicle is derived begins to grow (gonadotropin dependent) about day 1 of the menstrual cycle. The number of follicles in a cohort is unknown but is typically reduced to the ovulatory quota (unity in women and these monkeys) by the mid-follicular phase.

Another follicle will become dominant and a normal follicular phase is completed without delay. If the dominant follicle has already been selected, the entire process of recruitment must be reinitiated and ovulation will not occur until approximately 14 days after follicular ablation.[27]

The evidence for selection of a dominant follicle may also be defined endocrinologically. During recruitment, follicles in both ovaries actively grow and secrete E_2. Correspondingly, the venous effluent from each ovary contains similar concentrations of E_2. At the time of selection of the dominant follicle, E_2 secretion becomes asymmetric, with the venous effluent from the ovary that will eventually ovulate containing higher E_2 concentrations.[14] There are also differences in the intraovarian milieu. Follicles destined to become atretic contain decreased levels of E_2 and P and increased levels of androgens relative to the dominant follicle.

DOMINANCE

The interval of growth preceding ovulation but following selection is called dominance. The dominant follicle controls the endocrine milieu as it prepares itself, the reproductive tract, and the hypothalamic-pituitary axis for ovulation.

Follicular growth continues during dominance with enlargement of the antrum of the follicle and proliferation of the granulosa and the thecal layers. LH and FSH receptors are upregulated by the combined effects of E_2 and FSH as the follicle prepares itself for the LH/FSH surge and eventual ovulation.

The granulosa cells secrete increasing quantities of estrone and E_2. This estrogen effect is critical in coordinating the development of the different portions of the reproductive tract. The hypothalamic-pituitary axis requires E_2 priming of approximately 200 pg/mL for at least 36 hours to develop the ability to discharge and to surge LH sufficiently for ovulation.[40] Although the specific degree of priming that is necessary is unknown, the endometrium also requires E_2 priming in order to be able to respond appropriately to the secretion of P during the luteal phase. Similarly, estrogen stimulation of the endocervix and fallopian tube are required for normal gamete and embryo transport.[37] Therefore, the secretory products of the developing follicle prepare and synchronize the entire reproductive system for ovulation, fertilization, and implantation.

MIDCYCLE DYNAMICS

As midcycle approaches (Fig. 6), a number of physiologic processes stimulate the final maturational changes within the follicle and induce

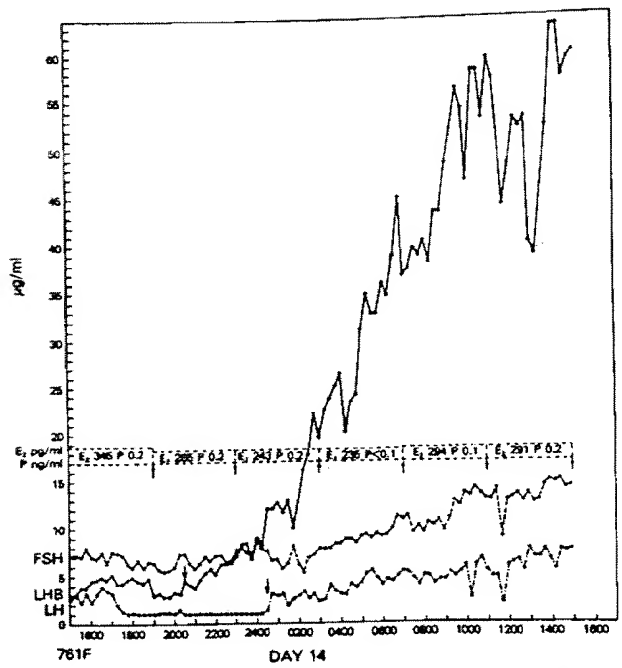

Figure 6. Plasma concentrations of FSH determined by RIA and of LH quantitated by RIA and in vitro bioassay on day 14 of the menstrual cycle when estradiol concentrations were approximately 300 pg/mL (periovulatory). Estradiol (E_2) and progesterone (P) concentrations for samples pooled over 4 hours are presented in the center of the figure.

ovulation. The precise means by which the oocyte communicates (if it does) that it is ready for ovulation is poorly understood.

Among the most prominent physiologic changes during midcycle is the LH surge. The greatly increased LH levels within the follicle stimulate three major events:

1. **Resumption of Meiosis.** Although the oocyte grows several fold after departing the resting state, the chromosomes remain in the diplotene state of prophase I. LH stimulates the resumption of meiosis with germinal vesicle breakdown and subsequent extrusion of the first polar body. At the time of ovulation, the oocyte progresses through metaphase of the second meiotic division. Timing of this process probably is not a stimulatory event but rather a release from prior inhibition. An ovarian peptide termed oocyte maturation inhibitor (OMI), acting in a paracrine or even autocrine fashion, has been postulated as the agent responsible for preventing early maturation of the oocyte.[65] OMI has not been isolated or characterized in the human or subhuman primates.
2. **Luteinization.** Following the LH surge, the follicular stromal cells convert from principally estrogen and protein secretion to secretion of E_2 and P. This rapid increase in P is responsible for inducing and coordinating several of the physiologic changes seen elsewhere in the reproductive system. This includes alteration of gonadotropin-releasing hormone (GnRH) pulsatility from the hypothalamus and release of LH from the pituitary, the onset of secretory change within the endometrium, and conversion of cervical mucus from a thin estrogenic state to a thick progestational state.
3. **Oocyte extrusion.**

MECHANISM OF OVULATION

One of the paramount events at midcycle is the gonadotropin surge. This accounts for resumption of meiosis, luteinization of the granulosa and theca cells with increased production of progesterone, and associated follicular events that culminate in ovulation with extrusion of a mature oocyte about 36 hours after the beginning of the LH surge.[51]

Several mechanisms may be involved in the aforementioned events.

Proteolytic Digestion of the Follicular Wall

In response to the gonadotropin surge, the content of plasminogen activator (PA) in follicular fluid and granulosa cells increases.[13, 25, 35, 64] There is still some controversy whether FSH is a more potent stimulant of PA production than LH or whether both gonadotropins have the same effect.[6] This action of gonadotropin is probably modulated by estrogens and P.[66] In preovulatory rat follicles in culture, tissue PA (tPA) and urokinase-type PA (u-PA) were produced, but only t-PA was stimulated by gonadotropins.[55] The increase in tPA produces a parallel increase in intrafollicular plasmin content (and other serine proteases), which weakens the follicular wall and probably produces activation of

latent collagenase.[24, 73] The activity of these enzymes is regulated by collagenase inhibitors that have been found in different species, including humans.[9-11] These inhibitors act together to control the site and extent of tissue remodeling that occurs during ovulation.[1]

Action of Other Mediators

Prostaglandins are definitely involved in follicular rupture and extrusion of the oocyte, although the nature of their action is not completely understood. Indomethacin in vivo can inhibit ovulation in rabbits and other species with production of luteinized unruptured follicles,[39] and this inhibition can be reversed by exogenous administration of prostaglandins.[58] Prostaglandin E_2 (PGE_2), prostaglandin F_2-α (PGF_2-α) and prostaglandin I_2 (PGI_2) are increasingly produced under human chorionic gonadotropin (hCG) stimulation.[42] It was proposed that follicular rupture may be achieved through vascular changes induced by PGI_2 within the follicular wall.[72]

Histamine, which is found in the ovarian hilum around the vessels[41] and which is capable of inducing follicular rupture but with less efficiency than hCG, is probably involved in ovulation.

Bradykinin, a nonpeptide released at inflammatory sites by cleavage of kininogens by a serine protease (kallikrein), also probably modulates ovulation,[3] by stimulation of prostaglandin synthesis and ovarian contractility and activation of collagenases directly or via PA.[63]

Mucification

FSH and LH stimulate the production and deposition of hyaluronic acid, a nonsulfated glycosaminoglycan, around the oocyte within the corona radiata, which disperses the cumulus and separates the oocyte-cumulus complex from the granulosa membrane. This facilitates the extrusion of the oocyte at the time of follicular rupture.[17] Cumulus expansion is induced in vitro by FSH.[15] The expansion is mediated by substances secreted by the oocyte itself.[5, 57] Apparently, serum (or follicular fluid) is necessary for the accumulation of hyaluronic acid in the cumulus matrix.[56]

Follicular fluid also contains sulfated glycosaminoglycans that inhibit hyaluronic acid synthesizing activity by cumulus cells.[18] It was hypothesized that the function of these sulfated glycosaminoglycans may be to inhibit precocious cumulus expansion which could result from the FSH present in antral follicles prior to the midcycle gonadotropin surge. Glycosaminoglycans also may prevent early demise of the oocyte after ovulation until fertilization is achieved[59] and are also probably involved in vascular changes in the follicle.[60]

Muscle Activity

Smooth muscle was found in the follicular wall of different species, including humans,[50] and changes in ovarian contractility have been observed spontaneously or after stimulation.[43, 62, 71] Perhaps the function of this activity is to maintain a constant tension on the follicular wall, thereby assisting in the rupture and facilitating the extrusion of the oocyte and follicular collapse.

Conversion from Avascular to Vascularized Status

Before follicular rupture, the granulosa membrane is avascular. Vessels are limited to the thecal compartment. Vascularization of the granulosa begins at ovulation and is maximal by postovulatory day 8 or 9.[28] Gospodarowicz found that the corpus luteum and the luteinized granulosa cells produce a substance capable of inducing angiogenesis. The change from an avascular to a vascular status is important for the delivery of lipoproteins and other substrates to the luteal cells.

There is probably more than one angiogenic factor in the follicle. Injection of ovarian extracts induce blood vessel formation when applied to the cornea of mice.[56] Human follicular fluid has been found to have angiogenic capability.[23] It has been demonstrated that angiotensin II has angiogenic activity, although it is not known whether this action is direct or is mediated by other substances such as prostaglandins or by cellular mechanisms.[20] The presence of a high level of renin and proteases, which have the ability to cleave angiotensinogen and generate angiotensin I, have been demonstrated in follicular fluid. The renin-like activity in follicular fluid was found to be 15 times higher than in plasma drawn simultaneously in women undergoing ovulation stimulation with gonadotropins,[21] but not in follicular fluid from nonstimulated women.[2]

Cytokines, glycopeptide signal mediators secreted by mononuclear phagocytes, polymorphonuclear cells, and various other cells that act in a paracrine or autocrine fashion seem to have a central role in the control of protease production and activation, and in turn proteases have an important role in angiogenesis.[46] Human follicular fluid contains tissue macrophages, and there is a high level of interleukin-1 (IL-1) in follicular fluid.[44] IL-1 has been shown to stimulate the de novo secretion and production of latent collagenase, gelatinase, and proteo-

glycanase in some tissues, and to decrease the production of tissue inhibitor of metalloproteinases. It also affects the production of PA, probably through mediation of prostaglandin E_2 synthesis. These proteases degrade tissue components, enabling the endothelial cells to migrate and proliferate to form new vessels.[46] Vascular endothelial growth factor, a newly described angiogenic factor, is expressed in rat corpus luteum cells but not in granulosa cells. Transforming growth factor-β, which was isolated from porcine follicular fluid,[4] also has angiogenic properties.[48, 52]

LUTEAL FUNCTION

After expulsion of the oocyte-cumulus complex, the granulosa and theca cells that remain within the follicle convert from the production of E_2 and follicular peptides to the production of E_2 and P.[35] This process, termed luteinization, actually begins prior to ovulation but requires the LH surge for completion.

Luteal function is dependent both qualitatively and quantitatively on normal development of the granulosa and theca cells during the preceding follicular phase. Inadequate proliferation of these gonadal stromal cells during the follicular phase or incomplete luteinization during the early luteal phase results in decreased secretion of E_2 and P. This in turn may cause altered function of the fallopian tube and endometrium, possibly resulting in abnormal gamete or embryo transport and decreased opportunities for implantation success.

The corpus luteum is not an autonomously functioning unit, but is dependent on continued LH support, at least for 4 to 5 days after ovulation.[36] Several experiments have demonstrated that removal of LH support through medical or surgical hypophysectomy or neutralizing antibodies to LH results in decreased P production and shortened luteal phases.[7, 30, 67] Pulsatile gonadotropin stimulation is not required for maintenance of the corpus luteum. This has been documented by the ability to maintain corpus luteum function with the tonic stimulation provided by an intramuscular injection of hCG. However, typically progesterone release from the corpus luteum is pulsatile and corresponds to the endogenous gonadotropin pulses.[22, 31]

The corpus luteum maintains its ability to secrete E_2 and P for several weeks if adequate LH or hCG stimulation is provided. The hCG stimulation seen during the first trimester of pregnancy maintains steroid production well beyond the seventh gestational week, when placental steroid production becomes adequate to maintain the pregnancy even in the absence of ovaries or exogenous hormonal support.[8, 33, 45]

CLINICAL CORRELATES OF OVULATION INDUCTION

Ovulation induction with clomiphene citrate or injectable gonadotropins is used commonly in the treatment of a wide variety of clinical disorders. It is well established that coordinating the onset and duration of these therapies with the status of the developing cohort of follicles is helpful in patient management. Therapy is initiated during recruitment and is maintained for variable intervals. In general, feedback relationships in these patients will produce a spontaneous LH surge so that no specific monitoring or therapeutic intervention is required. However, some patients may have an attenuated surge or none at all. In these cases, it may be necessary to administer hCG as a surrogate for the LH surge. The timing of hCG administration is based on clinical parameters such as plasma $E_2 > 250$ pg/mL and follicular diameter (usually 20 to 22 mm).[12]

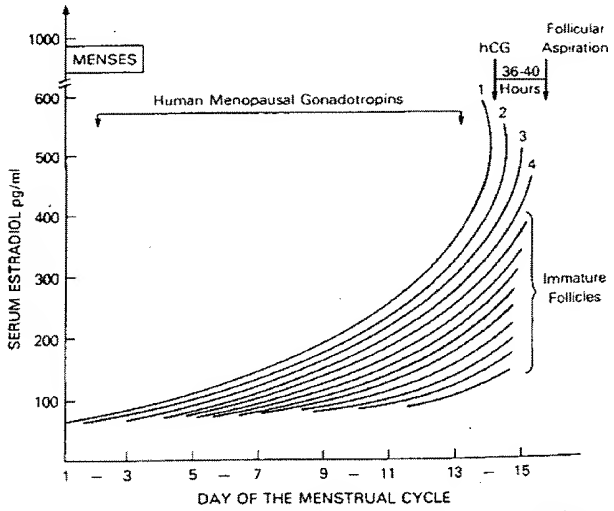

Figure 7. Human menopausal gonadotropin-stimulated follicular maturation overrides selection of a single dominant follicle in the natural cycle. Note that only a few follicles can be regarded as developing quasisynchronously. If human chorionic gonadotropin (hCG) is given too late, the most advanced follicles may yield post-mature eggs of low viable potential.

In contrast to clomiphene citrate, therapy with injectable gonadotropins is begun earlier in the follicular phase, typically on cycle day 2 or 3. A greater number of follicles are still available for recruitment at this time. This, along with the increased concentrations of gonadotropins, results in ongoing development of multiple follicles. Initiating gonadotropin therapy later in the cycle decreases the number of quasisynchronous follicles available for recruitment. This is consistent with the declining number of follicles remaining in the cohort that have not begun the process of atresia. It should be noted however, that if gonadotropin levels are maintained sufficiently high other smaller follicles may be recruited as they enter the stage of gonadotropin sensitivity. These smaller follicles lag behind the follicles that were recruited earlier and are unlikely to produce fertilizable oocytes and thus provide no clinical benefit (Fig. 7).

The duration of gonadotropin administration in these patients is variable, and specific monitoring is required to direct therapy. The elevated gonadotropin concentrations tend to accelerate development, and follicular maturity becomes adequate to produce mature oocytes by approximately cycle day 12.[30] The majority of these patients do not have timely spontaneous LH surges, possibly due to increased gonadal

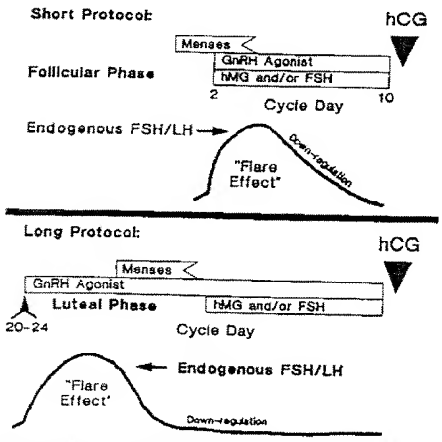

Figure 8. Two protocols are shown for gonadotropin-releasing hormone agonists plus gonadotropins. The long protocol is more versatile overall, whereas the short protocol can save money, provided treatment is effective.

peptide inhibition of the hypothalamic-pituitary axis. Therefore, hCG is administered as a surrogate LH surge. Follicular diameters for any given degree of maturity are smaller, reflecting an accelerated, shortened duration of the follicular phase. Administration of hCG typically occurs when the lead follicle is 16 to 18 mm in size, usually on cycle day 8 to 11.[61] These approaches are altered when GnRH agonists are used in conjunction with gonadotropin therapy.

Whether one should use the long or short agonist/gonadotropin protocol depends on many variables, including the stimulation regimen, monitoring system, patient population, clinician's expertise, and the protocol used in the egg/embryo laboratory.[36, 47] Data show that both can work either well or poorly. The long protocol is more versatile overall, and therefore is preferred by me. Also, there can be revival of the antecedent corpus luteum in demise when, in the short protocol, LH rises re-elevate progesterone during initial treatment, potentially confounding the endometrium. With regard to cost, the short protocol can save money, provided treatment proves effective. The short protocol combines endogenous FSH/LH from the initial flare effect with the injected gonadotropin (Fig. 8).[34]

References

1. Beers WH: Follicular plasminogen and plasminogen activator and the effect of plasmin on ovarian follicular wall. Cell 6:379, 1975
2. Brameld JM, Broughton PF, Pipkin F, et al: Studies on the human ovarian renin-angiotensin system: Optimization of assay methodology and effects of follicular stimulants. J Endocrinol 127:513, 1990
3. Brannstrom M, Hellberg P: Brandykinin potenetiates LH-induced follicular rupture in the rat ovary perfused in vitro. Hum Reprod 4:475, 1989
4. Brown MR, Vaughan J, Hexum TD, et al: Endothelin releasing activity in calf serum and porcine follicular fluid. Biochem Biophys Res Commun 173:807, 1990
5. Buccione R, Vanderhyden BS, Caron PJ, et al: FSH-induced expansion of the mouse cumulus oophorus in vitro is dependent upon specific factor(s) secreted by the oocyte. Dev Biol 138:16, 1990
6. Canipari R, Strickland S: Studies on the hormonal regulation of plasminogen activator production in the rat ovary. Endocrinology 118:1652, 1986
7. Collins RL, Sopelak VM, Williams RF, et al: Induction of luteolysis and menstruation by a GnRH antagonist in primates [abstract #113]. *In* Programs and Abstracts of the 31st Annual Meeting of the Society for Gynecologic Investigation, San Francisco, 1984, p 86
8. Csapo AI, Pulkkinen MO, Ruttner B: The significance of human corpus luteum in pregnancy maintenance. I. Preliminary studies. Am J Obstet Gynecol 112:1061, 1972
9. Curry TE, Dean DD, Woessner JF, et al: The extraction of a tissue collagenase associated with ovulation in the rat. Biol Reprod 33:981, 1985
10. Curry TE, Sanders SL, Pedigo NG, et al: Identification and characterization of metalloproteinase inhibitor activity in human ovarian follicular fluid. Endocrinology 123:1611, 1988
11. Curry TE, Mann JS, Estes RS, et al: α_2-Macroglobulin and tissue inhibitor of metalloproteinases: Collagenase inhibitors in human preovulatory ovaries. Endocrinology 127:63, 1989

12. DeCherney AH, Laufer N: The monitoring of ovulation induction using ultrasound and estrogen. Clin Obstet Gynecol 27:993, 1984
13. Deutinger J, Kirchheimer JC, Reinthaller A, et al: Elevated tissue type plasminogen activator in human granulosa cells correlates with fertilizing capacity. Hum Reprod 3:597, 1988
14. DiZerega GS, Hodgen GD: Folliculogenesis in the primate ovarian cycle. Endocr Rev 2:27, 1981
15. Eppig JJ: Gonadotropin stimulation of the expansion of cumuli oophori isolated from mice: General conditions for the expansion in vitro. J Exp Zool 208:345, 1979
16. Eppig JJ: Regulation of cumulus oophorus expansion by gonadotropins in vivo and in vitro. Biol Reprod 23:545, 1979
17. Eppig JJ: Regulation of cumulus oophorus expansion by gonadotropins in vivo and in vitro. Biol Reprod 23:545, 1979
18. Eppig JJ, Ward-Bailey PF: Sulfated glycosamineglycans inhibit hyaluronic acid synthesizing activity in mouse cumuli oophori. Exp Cell Res 150:459, 1984
19. Erickson GF: Normal ovarian function. Clin Obstet Gynecol 21:31, 1978
20. Fernandez LA, Twickler J, Mead A: Neovascularization produced by angiotensin II. J Lab Clin Med 105:141, 1985
21. Fernandez LA, Tarlatzis BC, Caride VJ, et al: Renin-like activity in ovarian follicular fluid. Fertil Steril 44:219, 1985
22. Filicori M, Butler JT, Crowley WF: Neuroendocrine regulation of the corpus luteum in the human: Evidence for pulsatile progesterone secretion. J Clin Invest 73:1638, 1984
23. Frederick JL, Shimanuki T, DiZerega G: Initiation of angiogenesis by human follicular fluid. Science 224:389, 1984
24. Fukumoto M, Yajima Y, Okamura H, et al: Collagenolytic enzyme activity in human ovary: An ovulatory enzyme system. Fertil Steril 36:746, 1981
25. Galway AB, Lapolt PS, Tsafriri A, et al: Recombinant follicle-stimulating hormone induces ovulation and tissue plasminogen activator expression in hypophysectomized rats. Endocrinology 118:1652, 1986
26. Ganther G: Olfactogenital dysplasia at puberty. Acta Neuroveg (Wien) 21:345, 1960
27. Goodman AL, Hodgen GD: Between ovary interaction in the regulation of follicle growth, corpus luteum function, and gonadotropin secretion in the primate ovarian cycle. I. Effects of follicle cautery and hemiovariectomy during the follicular phase in cynomolgus monkeys. Endocrinology 104:1304, 1979
28. Gospodarowicz D, Thakral KK: Production of a corpus luteum angiogenic factor responsible for proliferation of capillaries and neovascularization of the corpus luteum. Proc Natl Acad Sci USA 75:847, 1978
29. Greep RO (ed): Recent Progress in Hormone Research. New York, Academic Press, 1983, p 1
30. Groff TR, Raj HGM, Talbert LM, et al: Effects of neutralization of luteinizing hormone on corpus luteum function and cyclicity in *Macaca fascicularis*. J Clin Endocrinol Metab 59:1054, 1984
31. Healey DL, Schenken RS, Lynch A, et al: Pulsatile progesterone secretion: Its relevance to clinical evaluation of corpus luteum function. Fertil Steril 41:114–121, 1984
32. Hodgen GD: The dominant ovarian follicle. Fertil Steril 38:281, 1982
33. Hodgen GD: Surrogate embryo transfer combined with estrogen-progesterone therapy in monkeys. JAMA 250:2167, 1983
34. Hodgen GD: Uses of GnRH analogs to prepare for IVF treatment cycles (also GIFT and related assisted reproductive technologies). Contemp Obstet Gynecol 35:10, 1990
35. Hsueh AJW, Adashi EY, Jones PDC, et al: Hormonal regulation of the differentiation of culture ovarian granulosa cells. Endocr Rev 5:76, 1984
36. Hutchinson JS, Zeleznik AJ: The rhesus monkey corpus luteum is dependent on pituitary gondaotropin secretion throughout the luteal phase of the menstrual cycle. Endocrinology 115:1780, 1984
37. Jansen RPS: Endocrine response of the fallopian tube. Endocr Rev 5:525, 1984
38. Katayma KP, Roesler M, Gunnarson C: Short-term use of gonadotropin-releasing hormone agonist (Leuprolide) for in vitro fertilization. J In Vitro Fert Embryo Transfer 5:332, 1989

39. Katz E, Dharmarajan AM, Sueoka K, et al: Effects of systemic administration of indomethacin on ovulation, luteinization, and steroidogenesis in the rabbit ovary. Am J Obstet Gynecol 161:1361, 1989
40. Knobil E: The neuroendocrine control of the menstrual cycle. Recent Prog Horm Res 36:53, 1980
41. Krishna A, Terranova PF: Alterations in mast cell degranulation and ovarian histamine in the proestrus hamster. Biol Reprod 32:1211, 1985
42. LeMaire WJ, Leidner R, Marsh JM: Pre- and post-ovulatory changes in the concentration of prostaglandins in rat Graafian follicles. Prostaglandins 9:221, 1975
43. Lipner HJ, Maxwell BA: Hypothesis concerning the role of follicular contractions on ovulation. Science 131:1737, 1960
44. Loukides JA, Loy RA, Edwards R, et al: Human follicular fluids contain tissue macrophages. J Clin Endocrinol Metab 71:1363, 1990
45. Lutjen P, Trounson A, Leeton J, et al: The establishment and maintenance of pregnancy using in vitro fertilization and embryo donation in a patient with primary ovarian failure. Nature 307:174, 1984
46. Masure S, Opdenakker G: Cytokine-mediated proteolysis in tissue remodelling. Experientia 45:542, 1989
47. Meldrum DR, Wisot A, Hamilton F: Timing of initiation and dose schedule of leuprolide influence the time course of ovarian suppression. Fertil Steril 50:400, 1988
48. Merwin JR, Anderson JM, Kocher O, et al: Transforming growth factor beta-1 modulates extracellular matrix organization and cell-cell junctional complex formation during in vitro angiogenesis. J Cell Physiol 142:117, 1990
49. Moon YS, Tsang BK, Simpson C, et al: 17β-Estradiol biosynthesis in culture granulosa and theca cells of human ovarian follicles: Stimulation by follicle-stimulating hormone. J Clin Endocrinol Metab 47:263, 1978
50. Okamure H, Virutamasen P, Wright KH, et al: Ovarian smooth muscle in the human being, rabbit, and cat. Am J Obstet Gynecol 112:183, 1982
51. Pauerstein CJ, Eddy CA, Croxatto HD, et al: Temporal relationship of estrogen, progesterone and luteinizing hormone levels to ovulation in women and infra human primates. Am J Obstet Gynecol 130:876, 1978
52. Pepper MS, Montesano R, Orci L, et al: Transforming growth factor-beta modulates basic fibroblast growth factor–induced proteolytic and angiogenic properties of endothelial cells in vitro. J Cell Biol 3:743, 1990
53. Peters H, Himelstein-Braw R, Faber M: The normal development of the ovary in childhood. Acta Endocrinol 82:617, 1976
54. Peters H: Folliculogenesis in mammals. *In* Jones RE (ed): The Vertebrate Ovary. New York, Plenum Press, 1977, p 121
55. Reich R, Miskin R, Tsafrira A: Follicular plasminogen activator: Involvement in ovulation. Endocrinology 116:516, 1985
56. Salustri A, Yanagishita M, Hascall VC: Synthesis and accumulation of hyaluronic acid and proteoglycans in the mouse cumulus cell-oocyte complex during follicle-stimulation hormone-induced mucification. J Biol Chem 264:13840–13842, 1989
57. Salustri A, Yanagishita M, Haskall VC: Mouse oocytes regulate hyaluronic acid synthesis and mucification for FSH-simulated cumulus cells. Dev Biol 138:26, 1990
58. Sato E, Ishibashi T, Koide SS: Inducement of blood vessel formation by ovarian extracts from mice injected with gonadotropins. Experientia 38:1248, 1982
59. Sato E, Miyamoto H, Koide SS: Glycosamineglycans in porcine follicular fluid promoting viability of oocytes in culture. Mol Reprod Dev 26:391, 1990
60. Sato E, Miyamoto H, Koide SS: Hyaluronic acid-like substance from mouse ovaries with angiogenic activity. Z Naturforsch C 45:873, 1990
61. Scott RT, Rosenwaks Z: Ovulation induction for assisted reproduction. J Reprod Med 34:108, 1989
62. Scott RT, Hodgen GD: The ovarian follicle: Life cycle of a pelvic clock. *In* Adashi E (ed): Clinical Obstetrics and Gynecology. Philadelphia, JB Lippincott Company, 1990, p 551
63. Sith C, Perks AM: The effect of bradykinin on the contractile activity of the isolated rat ovary. Acta Endocrinol 106:387, 1984
64. Smith C, Perks AM: The kinin system and ovulation: Changes in plasma kininogens

and kiningforming enzymes in the ovaries and blood of rats with four days estrous cycles. Can J Physiol Pharmacol 61:736, 1983
64. Strickland S, Beer HW: Studies on the role of plasminogen activator in ovulation. J Biol Chem 251:5694, 1987
65. Tsafriri A, Dekel N, Bar-Ami S: The role of oocyte maturation inhibitor in follicular regulation of oocyte maturation. J Reprod Fertil 64:541, 1982
66. Tsafriri A, Abisogun AO, Reich R: Steroids and follicular rupture at ovulation. J Steroid Biochem 27:359, 1987
67. Vande Wiele RL, Vogumil H, Dyenfurth I: Mechanism regulating the menstrual cycle in women. Recent Prog Horm Res 26:63, 1970
68. Wallach EE, Bronson R: Exogenous PGF2α can reverse the inhibition of indomethacin on ovulation in rhesus monkeys. Prostaglandins 10:128, 1975
70. Wallach EE, Wright KH, Hamada Y: Investigation of mammalian ovulation with an in vitro perfused rabbit ovary preparation. Am J Obstet Gynecol 132:728, 1978
71 Wright KH, Wallach EE, Fromm E, et al: Studies of rabbit ovarian contractility using chronically implanted transducers. Fertil Steril 27:310, 1975
72. Yoshimura Y, Hosoi Y, Atlas SJ, et al: The effect of cAMP on hCG-induced ovulation, oocyte maturation and prostaglandins production. [abstract 97]. *In* Programs and Abstracts of the 33rd Annual Meeting of the Society for Gynecologic Investigation, Toronto, Canada, 1986, p 77
73. Yoshimura Y, Wallach EE: Studies of the mechanism(s) of mammalian ovulation. Fertil Steril 47:22, 1987

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OVULATION DETECTION

Kamran S. Moghissi, MD

Ovulation is the central event of the reproductive cycle. Anovulation and ovulatory disorder account for 20% to 25% of causes of reproductive failure. Documentation of ovulation is thus an important step in the evaluation of infertile women. Predicting or detecting the time of ovulation may be of equal importance when certain procedures such as artificial insemination are contemplated, or for the purpose of natural family planning. Definite proof of ovulation is pregnancy or recovery of an ovum from the oviducts. Direct observation of corpus luteum with the presence of a stigma by pelvic endoscopy or laparotomy may be considered strong evidence of ovulation. Presumptive evidence of ovulation may be obtained by steroid or gonadotropic hormone assays in the blood or urine or by peripheral changes in the reproductive tract and other sites associated with ovulation.

An understanding of hormonal events that control the ovulatory process is essential to appreciate the physiologic basis of many tests that have been devised for documentation and timing of ovulation. Maturation of ovarian follicles is effected by tropic action of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) secreted by the anterior lobe of the pituitary gland. Release of FSH and LH is, in turn, controlled by gonadotropin-releasing hormone (GnRH), which is produced by the hypothalamus in a pulsatile fashion. The pattern of FSH and LH secretion during the menstrual cycle has been established and is shown in Figure 1.[1, 23, 39, 48] The ovulatory phase of the menstrual cycle is characterized by a rapid and significant rise of LH, which culminates in the LH peak. Ovulation usually occurs within 16 to 24

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